

IN THE CLAIMS ✓

Please cancel claims 1-29 and 34.

30. (Amended) A method of inducing immunoprotection in a warm-blooded animal comprising administering to the animal a vaccine comprising a microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable when in the animal and non-viable when outside of the animal, the system comprising at least one of the following nucleic acid sequences

B1 (a) an essential gene, wherein expression of the essential gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal; and

(b) a lethal gene, wherein expression of the lethal gene is lethal to the cell and the lethal gene is expressed when the cell is outside of the animal but not when the cell is in the animal.

31. (Amended) The ~~method~~ of claim 30 wherein the system further [comprising]comprises an expression gene wherein the ~~expression~~ gene encodes an antigen.

B2 33. (Amended) The method of claim 30, wherein the [cell]vaccine is administered to mucosal surfaces of the animal.

Sub C4 36. (New) The method of claim 30, wherein the microbial cell is a member of the *Enterobacteriaceae*.

B3 37. (New) The method of claim 36, wherein the microbial cell is an avirulent *Salmonella*.

38. (New) The method of claim 37, wherein the avirulent *Salmonella* is an avirulent derivative of a pathogenic *Salmonella* that attaches to, invades and persists in the gut-associated lymphoid tissue or bronchial-associated lymphoid tissue.

Sub C5 39. (New) The method of claim 30, wherein the cell comprises both an essential gene and a lethal gene.

40. (New) The method of claim 39, wherein the essential gene is essential for metabolism, growth, cell wall integrity, or cell membrane integrity.

*Sub C 6*  
41. (New) The method of claim 40, wherein the essential gene encodes an enzyme which catalyzes the biosynthesis of the cell wall and its precursors.

42. (New) The method of claim 41, wherein the essential gene encodes an enzyme which catalyzes a step in the biosynthesis of diaminopimelic acid (DAP).

43. (New) The method of claim 42, wherein the essential gene is an *asd* gene.

*Sub C 7*  
44. (New) The method of claim 40, wherein the essential gene is selected from the group consisting of *dapA*, *dapB*, *dapC*, *dapD*, *dapE*, *dal*, *ddl*, *fab*, *fad*, *pls*, a gene encoding a modification methylase, a gene encoding a DNA ligase, a gene encoding a DNA gyrase, and a gene encoding a phospholipase.

*B3*  
*Cont.*  
45. (New) The method of claim 39, wherein the lethal gene is selected from the group consisting of a member of the *gef* gene family, a plasmid maintenance gene, a gene encoding a nuclease, a gene encoding a phospholipase, a gene encoding an endolysin, a gene encoding a holin, and a gene encoding a tRNA with a wrong codon.

46. (New) The method of claim 44, wherein the lethal gene is the combination of bacteriophage P22 lysis genes 13 and 19.

47. (New) The method of claim 35, wherein the replication gene is *polA*.

*Sub C 8*  
48. (New) The method of claim 30, wherein expression of the essential gene or the lethal gene is regulated by a trans regulatory element.

49. (New) The method of claim 48, wherein the trans regulatory element is selected from the group consisting of a repressor, an antisense RNA, and an RNA polymerase.

*Sub C 9*  
50. (New) The method of claim 30, wherein expression of the essential gene or the lethal gene is regulated by using promoters or regulatory elements that are regulated by temperature, or by other regulatory systems adapted to function in a temperature-dependent manner.

C9  
51. (New) The method of claim 50, wherein the essential gene is operatively linked to regulation selected from the group consisting of a *virB* promoter with a *virF* gene and promoter elsewhere in the cell, a *virF* positive activator in combination with promoters of a *yopH* gene or a *yadR* gene.

52. (New) The method of claim 50, wherein the essential gene is on an extrachromosomal vector.

53. (New) The method of claim 52, wherein the essential gene is regulated by a  $P_L$  or a  $P_R$  promoter with a *cI857* repressor.

54. (New) The method of claim 53, wherein the *cI857* repressor is operatively linked to a *P<sub>trc</sub>* promoter.

B3  
Cont  
55. (New) The method of claim 52, wherein expression of the lysis gene is regulated by a  $P_{22}P_R$  promoter operatively linked to a  $P_{22}$  *c2* gene, wherein the  $P_{22}$  *c2* gene is regulated by a  $P_L$  promoter, and wherein the cell further comprises a chromosomal *cI857* gene.

56. (New) The method of claim 55, further comprising an essential gene operatively linked to a  $P_L$  promoter.

57. (New) The method of claim 56, wherein the *cI857* repressor is inserted into an inactive chromosomal gene, wherein the inactive chromosomal gene is an inactive essential gene.

58. (New) The method of claim 57, wherein the microbial cell is an avirulent *Salmonella*.

59. (New) The method of claim 58, wherein the avirulent *Salmonella* is an avirulent derivative of a pathogenic *Salmonella* that attaches to, invades and persists in the gut-associated lymphoid tissue or bronchial-associated lymphoid tissue.

60. (New) The method of claim 59, wherein the avirulent *Salmonella* further comprises an inactive gene selected from the group consisting of *cya*, *crp*, *PhoP*, *phoQ*, *ompR*, *galE*, *cdt*, *htrA*, and a gene with a mutation that imposes a requirement for an aromatic amino acid or a vitamin.

61. (New) The method of claim 57, wherein the extrachromosomal vector comprises pMEG-104.